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# Stool DNA Isolation 96- Well Kit Product Insert

**Product # 65600**

Norgen's Stool DNA Isolation 96-Well Kit provides a fast, reliable and simple procedure for high throughput isolation of DNA from fresh, frozen and preserved stool samples collected using Norgen's Stool Nucleic Acid Collection and Transport Tubes. The universal protocol conveniently allows for the isolation of total genomic DNA from all the various microorganisms and host cells found in the stool sample simultaneously. The kit removes all traces of humic acid using a combination of chemical and physical homogenization and lysis. A simple and rapid spin column procedure is then used to further purify the DNA. The purified DNA is of the highest quality and is fully compatible with downstream PCR and NGS applications.

## Norgen's Purification Technology

Purification is based on 96-well column chromatography. The DNA is preferentially purified from other cellular components such as proteins without the use of phenol or chloroform. The purification is designed to be performed using centrifugation. The process involves first adding the stool sample and Lysis Buffer L to a provided Bead Tube and vortexing briefly to mix. Lysis Additive A is then added to the Bead Tube and the tube is vortexed in order to efficiently and rapidly homogenize the sample. The sample is then centrifuged, and the supernatant is transferred to a DNase-free microcentrifuge tube. Binding Buffer I is added, and the lysate is incubated for 10 minutes on ice. The lysate is then spun for 2 minutes to pellet any cell debris, the supernatant is collected, an equal volume of 70% ethanol is added to the lysate and the solution is loaded onto a 96-well plate. The bound DNA is then washed using the provided Binding Buffer C and Wash Solution A, and the purified DNA is eluted using the Elution Buffer B. The purified total DNA is free of all inhibitors, including humic acid, and can be used in sensitive downstream applications including PCR.

## Specifications

| Kit Specifications | |
|---|---|
| Binding Capacity Per Well | 50 µg |
| Maximum Loading Volume Per Well | 500 µL |
| Size of DNA Purified | All sizes |
| Maximum Amount of Starting Material:<br>Fresh or frozen stool<br>Preserved stool* | <br>200 mg<br>400 µl |
| Time to Complete 96 Purifications | 60 minutes |

***** *Preserved stool sample using Norgen's Stool Nucleic Acid Collection and Transport Tubes (Cat# 45650)*

## Advantages

- Fast and easy high throughput processing using centrifugation
- Isolate total DNA from a variety of stool types
- Isolate high quality total DNA for NGS and PCR applications

**Kit Components**

| Component | Product # 65600 (192 preps) |
|---|---|
| Lysis Buffer L | 2 x 105 mL |
| Lysis Additive A | 25 mL |
| Binding Buffer I | 25 mL |
| Binding Buffer C | 110 mL |
| Wash Solution A | 2 x 38 mL |
| Elution Buffer B | 30 mL |
| Bead Tubes | 200 |
| 96-Well Plate | 2 |
| 96-Well Collection Plate | 2 |
| 96-Well Elution Plate | 2 |
| Adhesive Tape | 4 |
| Product Insert | 1 |

**Storage Conditions and Product Stability**
All solutions should be kept tightly sealed and stored at room temperature. This kit is stable for 1 year after the date of shipment.

**Precautions and Disclaimers**
This kit is designed for research purposes only. Not intended for use in diagnostic procedures. Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These are available as convenient PDF files online at *www.norgenbiotek.com*.

**Binding Buffer C** contains guanidinium salts and should be handled with care. Guanidinium salts forms highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions.

**Customer-Supplied Reagents and Equipment**
You must have the following in order to use the Plant/Fungi DNA Isolation 96-Well Kit:

- Micropipettors and multichannel pipettes
- DNAse-free microcentrifuge tubes
- 70% ethanol
- 96%-100% ethanol
- Ice bath
- Bead beater equipment (e.g. OMNI BEAD RUPTOR).
- Centrifuge with rotor for 96-well plate assembly (such as Thermo Fisher IEC Centra CL3 series or Beckman GS-15R)

## Flowchart

Procedure for Purifying Total DNA using Norgen's Total DNA Purification 96-Well Kit

Add stool sample, Lysis Buffer L and Lysis Additive A to Bead Tube

↓ Vortex for 3 minutes.
Centrifuge. Transfer lysate.

Add Binding Buffer I.
Incubate for 10 minutes on ice.

**SPIN** Transfer lysate

Add Ethanol

↓

Bind to 96-well plate

**SPIN**

Wash once with Binding Buffer C
Wash twice with Wash Solution A

**SPIN**

Elute DNA with
Elution Buffer B

**SPIN**

**Purified Total DNA**

# Procedures

All centrifugation steps are performed at room temperature. The correct rpm can be calculated using the formula:

$$RPM = \sqrt{\frac{RCF}{(1.118 \times 10^{-5})\,(r)}}$$

where *RCF* = required gravitational acceleration (relative centrifugal force in units of g); *r* = radius of the rotor in cm; and *RPM* = the number of revolutions per minute required to achieve the necessary *g*-force.

***Notes Prior to Use***

- Ensure that all solutions are at room temperature prior to use. If necessary, warm to 65°C to redissolve any precipitates.
- Prepare a working concentration of the **Wash Solution A** by adding 90 mL of 96-100% ethanol (provided by the user) to the supplied bottle containing the concentrated **Wash Solution A**. This will give a final volume of 128 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- The volumes stated in each procedure for lysate preparation are the volumes required to prepare samples for each well of the 96-well plate.

**1. Lysate Preparation**

a. Add up to 200 mg of stool sample to a provided Bead Tube and add 1 mL of **Lysis Buffer L**. Vortex briefly to mix stool and Lysis buffer L.

   For stool samples that have been preserved using Norgen's Stool Nucleic Acid Collection and Transport Tubes (Cat# 45630 and 45660), add 400 µL of preserved sample to a provided Bead Tube and add 600 µL of **Lysis Buffer L**. Vortex briefly to mix stool and Lysis Buffer.

b. Add 100 µL of **Lysis Additive A** and vortex briefly.
c. Secure tube horizontally on a flat-bed vortex pad with tape, or secure the tube in any commercially available bead beater equipment (e.g. OMNI BEAD RUPTOR). Vortex for 5 minutes at maximum speed for a flat-bed vortexer or S=5.00, T=0:20, D=0:10 and C=02 program on OMNI BEAD RUPTOR.
d. Centrifuge the tube for 2 minutes at **14,000 RPM (~20,000 × g)**.
e. Transfer up to 600 µL of clean supernatant to a DNAase-free microcentrifuge tube (not provided).

   **Note:** A thin layer will be formed on the top of the clean supernatant. Carefully collect only the clean supernatant although small pieces of the layer will not affect the quality of the DNA

f. Add 100 µL of **Binding Buffer I**, mix by inverting the tube a few times, and incubate for 10 minutes on ice.
g. Spin the lysate for 2 minutes at **14,000 RPM (~20,000 × g)** to pellet any cell debris.
h. Using a pipette, transfer up to 500 µL of clean supernatant (avoid contacting the pellet or layer on the top of the supernatant) into a 2 mL DNAase-free microcentrifuge tube (not provided).
i. Add an equal volume of 70% ethanol (provided by the user) to the lysate collected above (100 µL of ethanol is added to every 100 µL of lysate). Vortex to mix. **Proceed to Step 2.**

**2. Binding DNA to 96-Well Plate**

a. Place the 96-Well Plate on top of a provided 96-Well Collection Plate

b. Apply up to 500 µL of the lysate mixed with the ethanol (from **Step 1**) into each well of the 96-Well Filter Plate. Centrifuge the assembly at maximum speed or 4,000 *x g* (~4,000 RPM) for 2 minutes.

**Note**: Depending on the starting material, a small quantity of precipitates may appear in the lysate-ethanol mix. No additional step is required to remove these precipitates prior to application to the wells

c. Discard the flowthrough. Reassemble the 96-Well Plate and the bottom plate.

**Note**: Ensure that all of the lysate from each well has passed through into the bottom plate. If the entire lysate volume has not passed, centrifuge for an additional 2 minutes.

**3. DNA Wash**

a. Apply 500 µL of **Binding Buffer C** to each well of the 96-Well Plate. Centrifuge the assembly at maximum speed or 4,000 *x g* (~4,000 RPM) for 2 minutes.

**Note:** Ensure the entire wash solution has passed through into the bottom plate by inspecting the 96-Well Plate. If the entire wash volume has not passed, centrifuge for an additional 2 minutes.

b. Discard the flowthrough. Reassemble the 96-Well Plate and the bottom plate.

c. Apply 500 µL of **Wash Solution A** to each well of the 96-Well Plate. Centrifuge the assembly at maximum speed or 4,000 x g (~4,000 RPM) for 2 minutes.

d. Discard the flowthrough. Reassemble the 96-Well Plate and the bottom plate.

e. Repeat steps **3c** and **3d** to wash 96-Well Plate for a second time.

f. Centrifuge the assembly at maximum speed or 4,000 *x g* (~4,000 RPM) for 10 minutes in order to completely dry the plate.

g. Gently pat the bottom of the 96-Well Plate on a paper towel to remove residual wash buffer. Ensure wash buffer is completely removed from the each tip of the 96 wells.

**4. DNA Elution**

a. Stack the 96-Well Plate on top of the provided 96-Well Elution Plate.

b. Add 100 µL of **Elution Buffer B** to each well of the 96-Well Plate and incubate for 1 minute at room temperature.

c. Centrifuge the assembly at maximum speed or 4,000 *x g* (~4,000 RPM) for 3 minutes.

**5. Storage of DNA**

Use the provided adhesive tape to seal the Elution Plate. The purified DNA samples may be stored at –20°C for a few days. It is recommended that samples be placed at –70°C for long term storage.

## Troubleshooting Guide

| Problem | Possible Cause | Solution and Explanation |
|---|---|---|
| Poor DNA Recovery | Wells of the plate have become clogged | Do not exceed the recommended amounts of starting materials. The amount of starting material may need to be decreased if the wells of the plate show clogging below the recommended levels. See also "Clogged Wells in Plate" |
| | An alternative elution solution was used | It is recommended that the Elution Buffer B supplied with this kit be used for maximum DNA recovery. |
| | 70% Ethanol was not added to the clean lysate | Ensure that the appropriate amount of 70% ethanol is added to the lysate before binding to the wells of the plate. |
| | Ethanol was not added to the Wash Solution A | Ensure that 90 mL of 96-100% ethanol is added to the supplied Wash Solution A prior to use. |
| | Low DNA content in stool sample used | Health condition or medication history might affect microorganisms in the stool sample, thus the expected yield of DNA will vary greatly from these different sources. |
| Clogged Wells in Plate | Too much input | Stool contains high level of fiber or undigested tissue could result in column clogging. Reduce the input volume or use preserved stool sample. |
| | Centrifuge temperature too low | Ensure that the centrifuge remains at room temperature throughout the procedure. Temperatures below 15°C may cause precipitates to form that can cause the wells to clog. |
| DNA does not perform well in downstream applications | PCR reaction conditions need to be optimized | Take steps to optimize the PCR conditions being used, including varying the amount of template, changing the source of *Taq* polymerase, looking into the primer design and adjusting the annealing conditions. |
| | DNA was not washed with the provided Binding Buffer C and Wash Solution A | Traces of salt from the binding step may remain in the sample if the plate is not washed 3 times in total with Binding Buffer C and Wash Solution A. Salt may interfere with downstream applications, and thus must be washed from the column. |
| | Ethanol carryover | Ensure that dry spin under the DNA Wash procedure is performed, in order to remove traces of ethanol prior to elution. Ethanol is known to interfere with many downstream applications. |

| Related Products | Product # |
|---|---|
| Stool DNA Isolation Kit | 27600 |
| Stool Nucleic Acid Collection and Transport Tubes (50 Tubes) | 45660 |
| Stool Nucleic Acid Isolation Kit | 45600 |
| Stool Total RNA Purification Kit | 49500 |
| Stool DNA Isolation Kit (Magnetic Bead System) | 55700, 63100 |
| Stool Nucleic Acid Collection and Preservation System | 63700 |

**Technical Support**
Contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362.

Technical support can also be obtained from our website (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

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